PARTICLE-BOUND THYMIDYLATE KINASE IN MOUSE LIVER, A POSSIBLE FACTOR IN THE CONTROL OF DNA SYNTHESIS

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Thymidylate kinase (TMP kinase) activities measured in resting mammalian tissues have been shown to be very low compared with activities in growing tissues and it is not entirely clear whether the low activities are a reflection of a low enzyme level or other complicating factors. Canellakis et al (1959) expressed the view that in adult rat liver a deficiency of the enzyme may limit DNA synthesis. In E. coli however, it has been shown that TMP kinase remains relatively constant throughout the growth cycle of the organism (Bessman, 1959). There have been suggestions of other reasons for the low activities measured in resting tissues. Hiatt and Bojarski (1960) found that after thymidine administration or addition of thymidine to the homogenizing medium, TMP kinase activity of rat liver extracts increased, an effect attributable at least in part, to enzyme stabilization (Hiatt and Bojarski, 1960). The possibility of dephosphorylating enzymes interfering with DNA synthesis has been investigated by Gray et al (1960). They found that although TMP kinase activity of rat liver could be increased by ammonium sulfate fractionation, there was poor correlation between dephosphorylating and inhibitory activities in the various fractions. In our work on the kinetics of thymidine-triphosphate synthesis (Kielley, in press), it was observed that synthesis started with a burst of activity, but quickly leveled off before significant uptake occurred. These characteristics were suggestive of a control mechanism rigidly suppressing synthesis. Further investigation of the problem of

TMP kinase in resting tissue has shown that the enzyme level in mouse liver is actually high, possibly higher than in growing tissue (hepatoma). This demonstration was achieved principally by the finding that a major part of the enzyme in mouse liver is bound to cell particles from which activity can be released.

Mouse liver was homogenized with 4 volumes of 0.05 M glycylglycine buffer (pH 7.4, containing 0.01 M potassium phosphate and 5×10^{-6} M TMP). The homogenate was centrifuged at 135,000 x g for 1 hr. to obtain a soluble supernatant fraction and a residue fraction. Relative TMP kinase activities of these tissue preparations are shown in Table 1. For comparison with a growing tissue, activities of some mouse ascites hepatoma preparations are included. More detail on the phosphorylation characteristics of the various tissue preparations are seen in time course experiments (Fig. 1). The fact that the soluble fraction of mouse liver showed higher and much more sustained activity than the unfractionated homogenate indicates that interfering factors were removed in the residue fraction. The residue fraction containing nuclei, mitochondria and microsomes, showed even less phosphorylating activity than the homogenate. Interference by the residue fraction may be due, at least in part, to the presence of TMP nucleotidase in microsomes (Fiala et al, 1962). The soluble fraction of liver obtained by high speed centrifugation of the homogenate represents to a large extent the enzyme source of tissue extracts which have been used in the past for studies of TMP kinase activity. As will be shown, the activity found in the soluble fraction accounts for only a fraction of the total TMP kinase of mouse liver.

Earlier experiments on the distribution of TMP kinase in isolated cell fractions of mouse liver (unpublished experiments of the author) indicated that enzyme activity could be detected only in the soluble fraction and in mitochondria. Intact mitochondria showed very little activity, but extracts made by freezing and thawing the particles often showed considerable activity. The residue fraction of mouse liver was

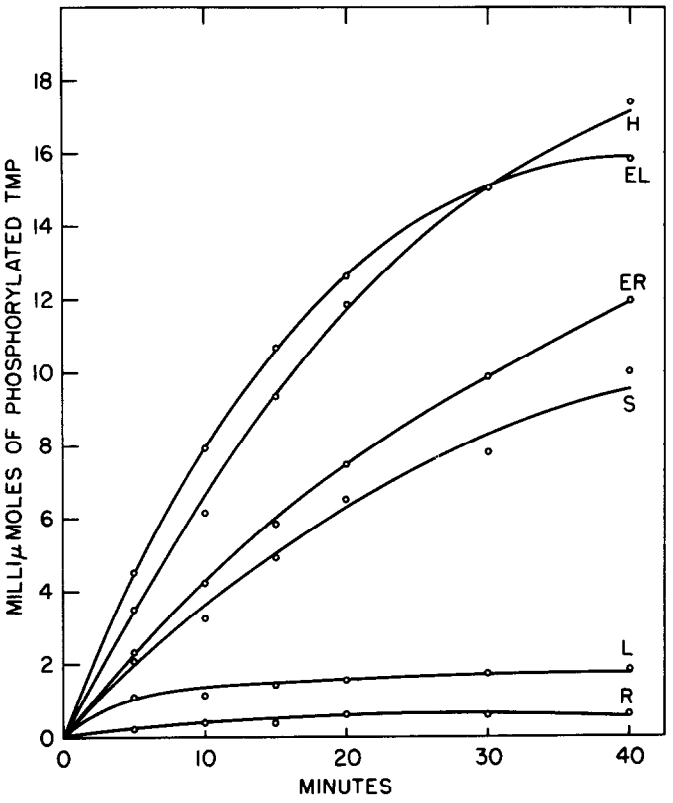

Fig. 1. Time course of TMP phosphorylation of mouse liver preparations and hepatoma.

L, liver homogenate; S, soluble fraction; R, residue fraction; H, hepatoma; EL, extract of frozen liver homogenate; ER, extract of frozen residue fraction.

Tissue preparations are described in the text. TMP kinase assay as described in Table 1.

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therefore investigated for total bound enzyme under conditions favoring maximum enzyme protection. The residue fraction obtained from the homogenate was resuspended to the original volume of the homogenate with the buffer medium and frozen at -20°. The preparation was thawed at 2° in the presence of added TMP (5 mμmoles of TMP per ml.) with occasional stirring to ensure mixing of the added TMP with the tissue. Alternate freezing and thawing were repeated 2 times adding TMP each time the preparation was thawed. The final suspension was centrifuged at 135,000 x g for 1 hr. The extract thus obtained from the residue fraction contained a large

amount of TMP kinase (Table 1) relatively unaccompanied by inhibitory activities (Fig. 1). It can be seen that the TMP kinase activity of mouse liver, taken as the sum of activities in the soluble fraction and that extracted from the residue fraction, nearly equals the activity found in hepatoma. Enzyme extracts of mouse liver prepared directly from the homogenate by freezing and thawing as described, contained more activity than the hepatoma (see extract of frozen homogenate, Table 1) and showed phosphorylation characteristics similar to those of hepatoma (Fig. 1).

Table 1. TMP Kinase in Mouse Liver Preparations

TMP Kinase assay: The incubation medium (0.25 ml.) pH 7.4, contained 10 μmoles tris-HCl buffer, 6 μmoles potassium phosphate, 4 μmoles $MgCl_2$, 2 μmoles ATP, 40 mμmoles TMP-2C^{14} (Schwarz, sp. activity, 2.5 x 10^5 cpm/μmole) and 0.05 ml. enzyme. After incubation, 0.5 ml. of water was added and the mixture heated in a boiling water bath for 2 min. The enzyme digest was treated with semen phosphatase according to Lehman et al (1958). The pH of the digest was adjusted to 7.5 with 0.1 N NaOH and the mixture heated in a boiling water bath for 2 min. Precipitated protein was centrifuged off and the clear digest was applied to a small DEAE cellulose column. After elution of thymidine with 0.005 M NH_4HCO_3, phosphorylated TMP was eluted with 0.5 M NH_4HCO_3 containing 1 M NH_4OH. After evaporating the solution and plating on a stainless steel planchet, radioactivity was determined in a gas flow counter.

Tissue Preparation	TMP Kinase Activity*	
	Liver	Hepatoma**
Homogenate	5.1	16.3
Soluble Fraction	7.7	14.7
Residue Fraction	1.0	
Extract of Frozen Residue	8.4	
Extract of Frozen Homogenate	18.9	

*Expressed as μ moles of TMP phosphorylated per hr. per mg. of tissue DNA-P and based on initial rates (5 min.) at 37° with 2.8 μg. of tissue DNA-P or its equivalent.

**No significant difference in the activities of fresh and frozen hepatoma was seen.

The demonstration that resting liver has potential TMP kinase activity comparable to activities found in growing tissue gives substantial support to the concept that cells whether growing or resting, maintain a rather constant level of the enzyme. Furthermore, the finding of particle-bound TMP kinase in mouse liver suggests that resting mammalian cells may control DNA synthesis through association of the enzyme with specialized cell structures to form an inactive enzyme complex.

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